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Identification of volatile organic compounds in the urine of patients with cervical cancer. Test concept for timely screening

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ABSTRACT

The objective of this research was to identify a global chemical pattern of volatile organic compounds (VOCs) in urine capable of discriminating between women with cervical cancer (CC) and control women using an electronic nose and to elucidate potential biomarkers by gas chromatography-mass spectrometry (GC–MS). A cross-sectional study was performed, with 12 control women, 5 women with CIN (Cervical Intraepithelial Neoplasia) and 12 women with CC. Global VOCs in urine were assessed using an electronic nose and specific by GC–MS. Multivariate analysis was performed: Principal Component Analysis (PCA), Canonical Principal Coordinate Analysis (CAP) and Partial Least Squares Discriminant Analysis (PLS-DA) and the test's diagnostic power was evaluated through ROC (Receiver Operating Characteristic) curves. Results from the PCA between the control group compared to the CC present variability of 98.4% ($PC_1 = 93.9\%$, $PC_2 = 2.3\%$ and $PC_3 = 2.1\%$). CAP model shows a separation between the overall VOCs profile of the control and CC group with a correct classification of 94.7%. PLS-DA indicated that 8 sensors have a higher contribution in the CC group. The sensitivity, specificity, value reached 91.6% (61.5%-99.7%) and 100% (73.5%-100%) respectively, according to the ROC curve. GC–MS analysis indicated that 33 compounds occur only in the CC group and some of them have been found in other types of cancer. In all, this study provides the basis for the development of an accessible, non-invasive, sensitive and specific screening platform for cervical cancer through the application of electronic nose and chemometric analysis.

1. Introduction

Cervical cancer (CC) is the third most common cancer in women worldwide [1], in 2020 half a million women suffered from cancer and about 342,000 women died, regarding cervical tumors, the World Health Organization (WHO) states that subtypes of human papillomaviruses (HPV) 16 and 18 are responsible for 70% of cervical cancer and pre-cancerous lesions of the cervix [2].

CC can be detected by a laboratory test that examines cervical cells obtained by a gynecological procedure called a Pap smear or by liquid-based cytology [3].

The early stages of CC involve the early growth of abnormal cells known as cervical dysplasia. Dysplastic cells resemble cancerous cells but are not considered malignant as long as they remain on the surface of the cervix and do not invade healthy tissue [4]. Studies have highlighted the importance of HPV screening, as women with normal cytology but infected with the virus can develop cancer [5]. In addition, a systematic review of visual inspection examination with cytology, acetic acid and colposcopy shows that for every 1000 examinations there are 20, 58 and 464 false-positive cases of advanced lesions (Cervical Intraepithelial

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Neoplasia (CIN) 2 and 3) respectively [6] and that the HPV-DNA screening test is more accurate than cytology in detecting CIN even in minor lesions [7].

While screening methods and vaccination programs are employed in developed countries, the disparity in the burden of disease in women in developing countries is more profound [8], this is reflected in mortality data where more than 80% of deaths occur in low- and middle-income countries [1], an important point is that women who are routinely screened are more likely to get early diagnosis and treatment.

Moreover, not all population groups may benefit equally from the proposed HPV and CC screening programs; there are reports that there is a higher incidence and mortality among racial and ethnic minority women, compared to their counterparts [9]. In addition, there are economic, social, psychological and cultural barriers, for example, some studies reveal women's discomfort with the Pap procedure as it involves an invasive pelvic examination to obtain cells from the cérvix by a clinician at clinics or hospitals, posing embarrassment, stress, anxiety, fear and inconvenience, and practical barriers such as time to result, clinic appointment and trust in the test [10].

Great efforts have been made to develop safe, reliable, non-invasive and inexpensive screening platforms. One very promising approach is the search for specific and reliable volatile biomarkers emitted by cancer cells, these biomarkers are the result of the modified metabolism of these cells, which are released in biological matrices and thus, can be detected. The focus of this discipline called *volatilomics* is the study of the differences between volatile organic compounds (VOCs) generated in normal cellular metabolism and pathophysiological conditions in various biological matrices (exhaled breath, blood, urine, among others), and through chemometric techniques selects the group of VOCs that can be used as biomarkers, thus, several investigations have shown their potential for screening of various diseases, including cancer [11–13]. Gas Chromatography coupled to Mass Spectrometry (GC–MS) has frequently been used for the identification of VOCs in cancer [14]. Another widely used methodology for the global identification of VOCs patterns uses gas sensors such as the electronic nose, this approach is based on an array of chemical sensors whose outputs are integrated by advanced signal processing to rapidly identify complex odor mixtures which are analyzed through multivariate statistical techniques that recognize patterns in the chemical vapors, therefore, can be used in the future to identify similar cases [15].

Considering the background, the objective of this research was to conduct an exploratory study to evaluate the feasibility of implementing the electronic nose for the identification of VOCs in urine samples and to elucidate potential biomarkers by gas chromatography coupled to mass spectrometry in groups of healthy women, women with HPV-infected cervical dysplasia and women with CC.

2. Materials and methods

2.1. Population and study design

The present work consists of an exploratory study, with a quantitative, descriptive, and experimental approach. Three study groups were defined: i) control group, healthy women negative for HPV 16 and 18 and normal cytology, ii) CIN group, women with a positive HPV test for HPV 16 and/or 18, and iii) CC group, women diagnosed with CC with a positive HPV test for HPV 16 and/or 18. The study was conducted on women from San Luis Potosí, México. Sample collection was carried out from January to May 2019, at the Hospital Dr Ignacio Morones Prieto of the Autonomous University of San Luis Potosí (UASLP). The eligibility criteria for HPV infected patients to participate in the study were as follows: i) Patients with cytological diagnosis of lesion type: L-SIL or H-SIL, according to Bethesda classification [16]; ii) Patients with a colposcopic diagnosis of L-SIL or H-SIL injury according to Bethesda classification [17]; (iii) signature of informed consent. The eligibility criteria for the Control group were as follows: i) Women with a Pap smear at least 6 months prior to the study, ii) Negative diagnosis of HPV infection; iii) signature of informed consent. The study elimination criteria were as follows: i) Impossibility of sample collection or insufficient sample; ii) Menstruation at sample collection; iii) Pregnancy present on the day of sample collection; iv) Recent urinary tract infections; v) No signature of informed consent. This protocol was authorized by the Ethics and Research Committee of the Faculty of Nursing and Nutrition of the UASLP, which was approved with the registration number CEIFE-2019–293. The proposed investigation in this protocol is under the Declaration of Helsinki.

2.2. Sample collection

The study participants were required to collect the first morning's urine under eight-hour fasting conditions at home, the sample was collected in sterile 50 mL polypropylene cups and then transported at 4 °C, after which aliquots were made for the determination of the urine VOCs fingerprint. All samples were frozen at −80 °C and analyze on the same week of sampling. The cervical smear sample was also collected for the genotyping analysis.

2.3. Histological technique

The Leica RM2125 RTS microtome was used to make the blades. The process is described as follows: i) At the beginning in the paraffin block a reduction was performed at the first level until the cervical tissue was visualized, then a reduction to the 30-μm paraffin block was performed, ii) Two cuts were taken from each biopsy, one of 5 μm for Hematoxylin-Eosine staining, and one of 30 μm for DNA extraction. iii) The slides (pre-filled) were immersed in the stretching bath, to locate the cuts. iv) When the cuts were in the slides, they went through a rehydration process using different percentage concentrations of ethyl alcohol (EtOH, CTR Scientific), which was of 50°, 60°, 70°, 80°, 90° and absolute. All concentrations were left for 10 min and then the 5-μm ones were stained.

2.4. Haematoxylin-Eosine staining technique

The slides were immersed in the stretching bath, the cuts were placed over them and introduced into a heat and drying chamber (Binder, ED-115, Germany) at 65 °C, to melt the paraffin. The slides were stained using the staining protocol chosen for the sample; the staining technique used was Hematoxylin-Eosine (Merck KGaA, 1050175, Germany) (Hycel, 686, Mexico) and then visualized on the double-head microscope (Leica Biosystems, DM-750, USA) at 10x and 40x.

2.5. Genotyping analysis: Determination of HPV 16 and HPV 18 by qPCR

The qPCR was performed using Thermo Scientific's PikoRealTM equipment with standardized oligonucleotides at the National Institute of Public Health, SSA for mRNA E6 HPV 16 and mRNA E6 HPV 18. The conditions for performing qPCR were as follows: i) temperature 95 °C, 10:00, ii) 40x cycles, iii) temperature 95 °C, 00:15; iv) temperature 60 °C, 01:00; v) temperature 95 °C, 00:15; vi) temperature 60 °C, 01:00; vii) data Adquisition; viii) temperature 95 °C, 00:15; ix) curve Melt 60 °C − 95 °C; x) data Adquisition

Each run of qPCR was mounted on a 96-well polypropylene plate (Corning TM). The runs were performed in triplicate, with all 40 DNA samples (10 ng/μL), one negative control, and one positive control. Everything was done using SYBR green (qPCR SYBR®). The primers used were: SENSE AATGTTCAGGACACAGG and ANTISENSE GTTGCTTGCAGTACACACATTC for HPV 16. Or SENSE ACCCTACAAGCTACGATCT and ANTISENSE ACCTCTGTAAGTCCAATACTGTC for HPV 18.

2.6. Determination of urine VOCs by electronic nose

The analysis for the detection of the VOCs fingerprint in urine was performed through the Cyranose 320 (Sensigent®, California, USA) electronic nose. This equipment contains 32 chemical nanosensors, which are specific for certain functional groups of the VOCs present in the sample, this specificity is given by the different adsorption properties of each sensor by its sensing layer composition (poly-vinyl butyral, polyvinyl acetate, polystyrene, and polyethylene oxide and conducting nanoparticles as black carbon and carbon nanotubes) which, upon contact with the VOCs, generates a change in electrical resistance and thus, resulting in several different signals with varying intensities for each sensor and VOCs pattern. The resistance changes across the array are captured as a digital pattern that is representative of the test smell, the overall response to a particular sample produces a "smell print" specific to a stimulus.

For sample processing, first, the samples were thawed to ambient temperature, afterwards, 2 mL of urine were placed into a headspace 20 mL vial and was hermetically sealed under a nitrogen gentle stream. Then samples were incubated at 65 °C for 40 min before analysis. The electronic nose setting consisted of a constant flow rate of 120 mL/min for 20 s of baseline recording with ultra-pure nitrogen and a sample recording period of 70 s and was subsequently increased to a flow rate of 180 mL/min of ultra-pure nitrogen for sample line purge and air inlet for 20 s, with a substrate temperature of 32 °C. As an internal quality control, the resistance (mΩ) of the 32 sensors was registered each day to eliminate the drifting and to assess the quality of the analysis (data not shown).

2.7. Identification of urine VOCs by GC–MS

The processing of the samples for the extraction of VOCs in urine was performed as follows, in brief, 2 mL aliquots of urine were taken and deposited in 20 mL headspace vials hermetically sealed with PTFE septa. Headspace-Solid Phase Microextraction (HS-SPME) was then performed, the SPME holder for manual sampling and fiber were purchased from Supelco (Aldrich, Bellefonte, PA, USA). The SPME device included a fused silica fiber coating with Polydimethylsiloxane (PDMS) of 100 μm. Prior to use, the SPME fibre was conditioned at 270 °C for 50 min in the GC injector, according to the manufacturer's recommendations. Then, the fiber was daily conditioned for 10 min at 250 °C. HS-SPME was performed by exposing the fiber to the HS in the sample vial for 40 min at 65 °C. After the extraction/concentration step, the SPME coating fiber was manually introduced into the GC–MS injection port at 270 °C for 5 min. Finally, samples were analyzed by gas chromatography (GC) (Agilent 6890) coupled to a mass spectrometry detector (MS) (Agilent 5975) in electron impact ionization mode (EI). The injection port was operated in splitless mode, its temperature was 250 °C; helium was used as carrier gas at a pressure of 36 psi with a constant flow of 0.9 mL/min. The chromatographic separation was carried through an HP 5MS (30 m × 0.25 mm × 0.25 μm) column (Agilent). The conditions of the oven settings were as follows: 95 °C (1 min), 195 °C (15 °C/min), 206 °C (2 °C/min) with hold until minute 13.2, then an increase to 320 °C (40 °C/min) and held to minute 24. The tune parameters were emission: 35 μA; energy: 69.9. SCAN mode (50–350 *m/z*) was employed to identify the volatile organic compounds urine fingerprint. The compounds were identified through the NIST 14 library. The results were obtained and processed using Chemstation software (Agilent®).

2.8. Statistical and chemometric analysis

Descriptive statistics of the study groups were performed, recording the mean and standard deviation of the anthropometric parameters using GraphPad Prism 9.0 ® software.

Multivariate analysis to evaluate the sensors that discriminate between groups was performed using MetaboAnalyst 5.0 statistical software. (https://dev.metaboanalyst.ca/MetaboAnalyst/home.xhtml) and PRIMER v7® statistical software with PERMANOVA ad in. The recorded data were the changes of the responses in the resistances (mΩ) of the 32 nanosensors, this obtained by the fractional difference given by **eq.** [1]:

$$\frac{\Delta R}{R_0} = \frac{(R_{max} - R_{0)}}{R_0} \quad [1]$$

Where:

R is the maximum resistance response of each sensor

R_o is the reference resistance of each sensor (ultra-pure nitrogen).

The sum was then normalized to reduce the environmental effect by dividing the response of each sensor by the sum of the absolute values of each sensor's response, based on **eq.** [2]

$$\left(\frac{\Delta R}{R_0}\right) i = \frac{\left(\frac{\Delta R}{R_0}\right) i}{\sum \left|\frac{\Delta R}{R_0}\right| j} \quad [2]$$

Where:

R is the maximum resistance response of each sensor

Ro is the reference resistance of each sensor (ultra-pure nitrogen).

$j = \frac{1}{\sum \left|\frac{\Delta R}{R_0}\right|}$

i = Correction constant for nanosensor drifting

An auto-scaling was performed to reduce the effects of the magnitude of the sensor responses by subtracting the mean of the samples from the individual sample response and dividing by the standard deviation of the samples.

The PCA was obtained through the multivariate data cloud that was the best to discriminate between predefined groups (CC, CIN, and controls). PCA provides a summary of all the variables entering in the statistical analysis by finding correlations among the variables. Following correlation, it reduces the variables into a smaller number of principal components which is responsible for the possible variance in the observed variables. Finally, PCA expressed the whole data sets in a global and qualitative visual pattern, highlighting similarities and differences between and within the sample [18]. Partial least squares discriminant analysis (PLS-DA) and variable importance in projection (VIP) were performed to identify the differential sensors among groups and to rank the sensor's response according to their importance in discriminating groups. PLS-DA is a supervised statistical method that uses multivariate regression techniques to extract, by a linear combination of independent variables, information to predict class belonging. It is used to improve separation between groups by rotating the PCA components to obtain a maximum separation.

All variables were transformed to a Euclidean distance dissimilarity matrix calculated from normalized (X- mean/standard deviation) and log (X + 1)-transformed data pre-processed from the 32-sensor ΔR data; the difference between groups was calculated using 9999 permutations [11,19]. Canonical Analysis of Principal Coordinates (CAP) was then used to ordinate the matrices, and to further determine the level of misclassification between sampling regions the method of leave-one-out was applied to variables in canonical space (using a K-fold of n = 27) to predict group associations and thus obtain the overall classification success rates, using a value of m = 3. Additionally, the CAP model was employed to predict the clustering of the new samples. The associations of the analyzed sensors with the CAP axis were evaluated by Spearman correlation.

Only the CAP1 axis was evaluated using the ROC curve (Receiver Operating Characteristic curve) because it represented 100% of the data. With a 95% confidence interval (CI) and the threshold value or cut-off point was selected with the highest specificity/sensitivity ratio [20].

For the analysis of urinary metabolite data identified by GC–MS, we used chromatographic peak area data and an identification quality greater than 70, then compared the presence of metabolites between the

study groups and through the Human Metabolome Database (https://hmdb.ca/metabolites) it was established the biological function between the compounds shared between the groups.

3. Results

The characteristics of the study participants are presented in Table 1. There were 29 women, of which there were, 12 control women, 5 women with CIN and 12 women with CC, in an age range of 27 to 51 years. The group of healthy women presented normal cytology and negative for HPV 16 and 18, the group of women with CIN 80% presented a classification of CIN 1 and 20% with CIN 2, regarding the group of women with CC 50% presented classification IIB, 16.6% with IA, IB and III. Patients with CIN presented only HPV 16 and patients with CC presented genotypes 16 and 18 in 100% of the samples.

Fig. 1**a** shows the plot of the 2 PCs of the PCA between the control group compared to CC, the analysis reaches an explanation of 98.4% of the variability using 3 PCs ($PC_1 = 93.9\%$, $PC_2 = 2.3\%$ and $PC_3 = 2.1\%$), a natural separation of the study groups between the control group and women with CC is observed. In Fig. 1**b**, the analysis of the 3 groups explains 96.2% of the variability of the data using 3 PCs ($PC_1 = 89.6\%$, $PC_2 = 4.4\%$ and $PC_3 = 2.2\%$).

Fig. 2**a** shows the PLS-DA values, the sample graph indicates the group belonging to each sample and the discrimination between the control group concerning the CC group can be visualized, a separation with the CIN group is not achieved. The variable importance in projection indicates that for the CC group the sensors S8, S31, S3, S20, S26, S14, S30, S7 present a greater contribution to the separation of the group, while for the controls the sensors S19, S18, S21, S17, S15 and S27, S27, S20, S26, S14, S30 and S7 present a greater contribution to the separation of the group (Fig. 2**b**).

The CAP model (Fig. 3**a**) displays a separation between the overall VOCs profile of the control and CC group with correct classification of 94.73 %, but not with the CIN group (Fig. 3**b**), there is a correlation with the CAP 1 axis with the variables of 0.75 and reaches a correct classification of up to 62 % ($p = 0.0038$). A Spearman correlation analysis was performed to establish the associations between CAP 1 and the sensors that correlate with the CAP axis, the results obtained indicate a positive significant correlation between S12, S15, S17, S18, S19, S21 and S22.

Furthermore, with the values created in the CAP 1 score, the cut-off point of −0.042 was established, which provided 91.6% sensitivity (confidence intervals at 95%: 61.5–99.7%) and 100 % specificity (confidence intervals at 95%: 73.5–100%) (Fig. 4).

Fig. 5 shows a representative GC–MS chromatogram of those obtained for each group. We identified 220 VOCs, 77 metabolites in the control group, 88 in the CIN group and 55 in the CC group, concerning the chemical groups, found, we report in the control group the presence in descending order of alcohols (24.6 %), alkanes (18.1 %), alkenes (9.0 %) and carboxylic acids (7.8 %), in the CIN group we reported alkanes (28.4 %), alcohols (26.1 %), alkenes (12.5 %), carboxylic acids (5.6 %) and esters (5.6 %), as for the CC group, we obtained alkanes (30.9 %), alcohols (23.6 %), alkenes (12.7 %), aldehydes (5.4 %) and esters (5.4 %). In the **supplementary material** (**Table S1, S2 and S3**) the representative compounds of each of the groups and the chemical family to which they belong are presented. Of the metabolites found, 57 unique compounds were for the control group, 66 for CIN and 33 for CC, 8 compounds were present in the control and CIN group, 7 compounds between control and CC and 9 compounds between CIN and CC and 5 compounds between the 3 groups (Fig. 6).

The compounds shared between the control group and CIN are 4,7,7-Trimethylbicyclo[2.2.1]hepta-2,5-diene; Androst-5-en-3-ol, 4,4-dimethyl-; (3beta)-; Azulene 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethyl)-; Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-; Humulane-1,6-dien-3-ol; Isocyclocitral; Octadecane; Tridecane, 4,8-dimethyl-, between the control group and CC are: 2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol; 6-Azaestra-1,3,5(10),6,8-pentaen-17-one, 3-methoxy-; Caryophyllene; Cyclopentanol; 3-methyl-2-(2-pentenyl)-, Hexadecane, 1-bromo-; Nonadecane; Thunbergol and the metabolites shared between the CIN and CC groups are Neoclovene-(I), dihydro-; (2,6,6-Trimethylcyclohex-1-enyl) acetic acid; 1-Heptatriacotanol; 1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene; 1-Naphthalenepropanol; alpha-ethenyldecahydro-alpha,5,5,8a-tetramethyl-2-methylene-; 2(1H)-Naphthalenone; octahydro-4a-phenyl-, trans-, 3,5,24-Trimethyltetracontane; Cyclohexanone 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethylidene)-; trans-, Phenol, 4,4′-(1,1-dimethyl-3-methylene-1,3-propanediyl) bis.

Table 1
Evaluated parameters in the study groups.

Parameters	Healthy	CIN	Cervical cancer
N	10	5	12
Age (years)	35.3 ± 1.7	35.2 ± 1.6	47.4 ± 2.4
Weight (kg)	59.8 ± 10.3	69.6 ± 14.8	65.3 ± 5.6
Height (m)	1.6 ± 0.04	1.5 ± 0.06	1.5 ± 0.06
BMI (kg/m^2)	23.0 ± 3.3	28.3 ± 5.9	26.5 ± 2.4
Smoking (N)	3	1	0
Alcohol consumption (N)	5	2	0
Sexual life onset (years)	17.4 ± 2.1	18.4 ± 1.1	18.4 ± 1.3
Number of sexual parthers	3 ± 1	2 ± 1	1.3 ± 0.4
Cq VPH16 (N)	0	5	12
Cq VPH18 (N)	0	0	12
Viral load (x10^6)	0	5.98 ± 4.79	6.58 ± 2.52
Pap smear (Yes, %)	100	100	100

4. Discussion

In this exploratory study, differences were found in the global VOCs between the control and CC groups by the electronic nose, identifying 8 sensors that influence the separation of the groups, and we were also able to determine unique volatile metabolites and those that are shared between the groups.

Urine profiles have been characterized and used for the diagnosis of diseases, the metabolites present are intermediate or end products of a large number of metabolic processes[21]. There are screening methods for HPV in urine, which are based on DNA methylation. These methodologies, while non-invasive, display low specificity/sensitivity [22], reducing the efficacy of CC screening programs.

Other methodologies for the screening of HPV and CC in urine have been described, such is the case of the study by Rajasekaran et al., who report that employing Steady-state and time-resolved fluorescence spectroscopy they were able to establish discrimination of CC patients (N = 60) from healthy subjects (N = 60) evaluating the biomarkers indoxyl sulfate, neopterin, and riboflavin, achieving a correct classification of 96.4% [23]. Nevertheless, the authors point out the low specificity of the metabolites found, since they are reported in other diseases, for example, indoxyl sulfate is a biomarker for kidney disease [24].

Another study in urine by Liang et al., conducted using Liquid Chromatography coupled to Time-of-Flight and Mass Spectrometry and multivariate data analysis, studying the metabolomic fingerprint in the urine of patients with CC and healthy women, identified a set of metabolites with strong discriminative power, such as 3-methylhistidine, citric acid, cytosine, indoleacetic acid, salicyluric acid, L-methionine, aminomalonic acid, glutaric acid, ursodeoxycholic acid and N-acetylornithine, which are involved in key metabolic pathways such as the citrate cycle, lysine degradation, tryptophan metabolism, cysteine and methionine metabolism [25]. In their study and in agreement with our results they state that the urine of CC patients reveals disease-specific metabolic imbalances. The study by Godoy-Vitorino et al., evaluated urine metabolites in patients with different HPV categories, including controls, HPV positive with simultaneous low and high-risk infections and HPV positive with high risk, their results were interesting. They indicated that the control and HPV-positive groups with low-risk

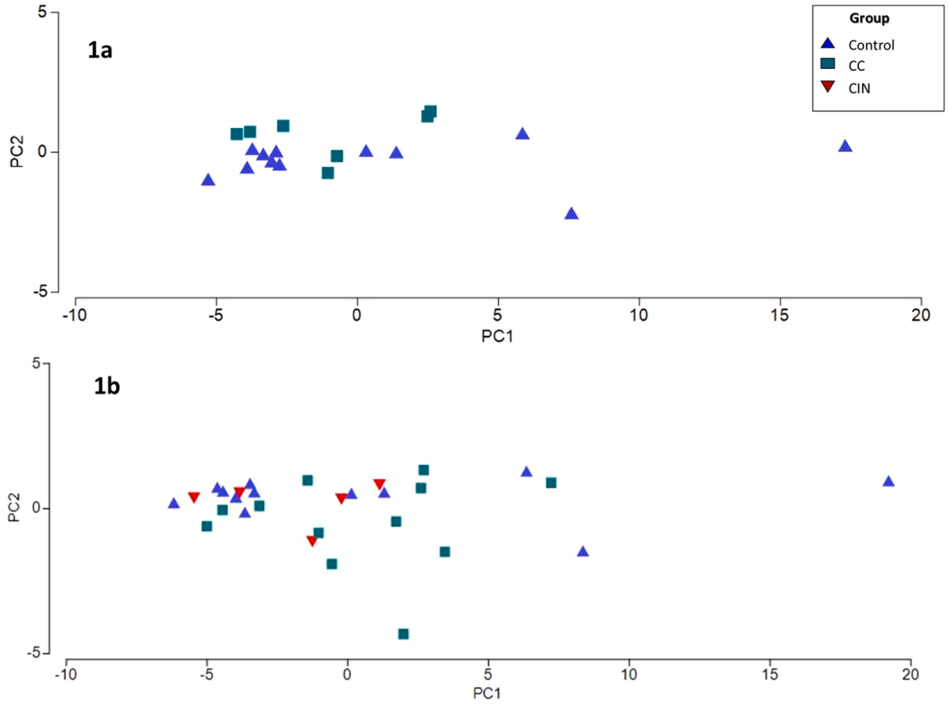

Fig. 1. Principal component analysis (PCA) of the studied groups. **1a)** Plot of Control group vs. cervical cancer; **1b)** plot of control group vs. CIN vs. cervical cancer.

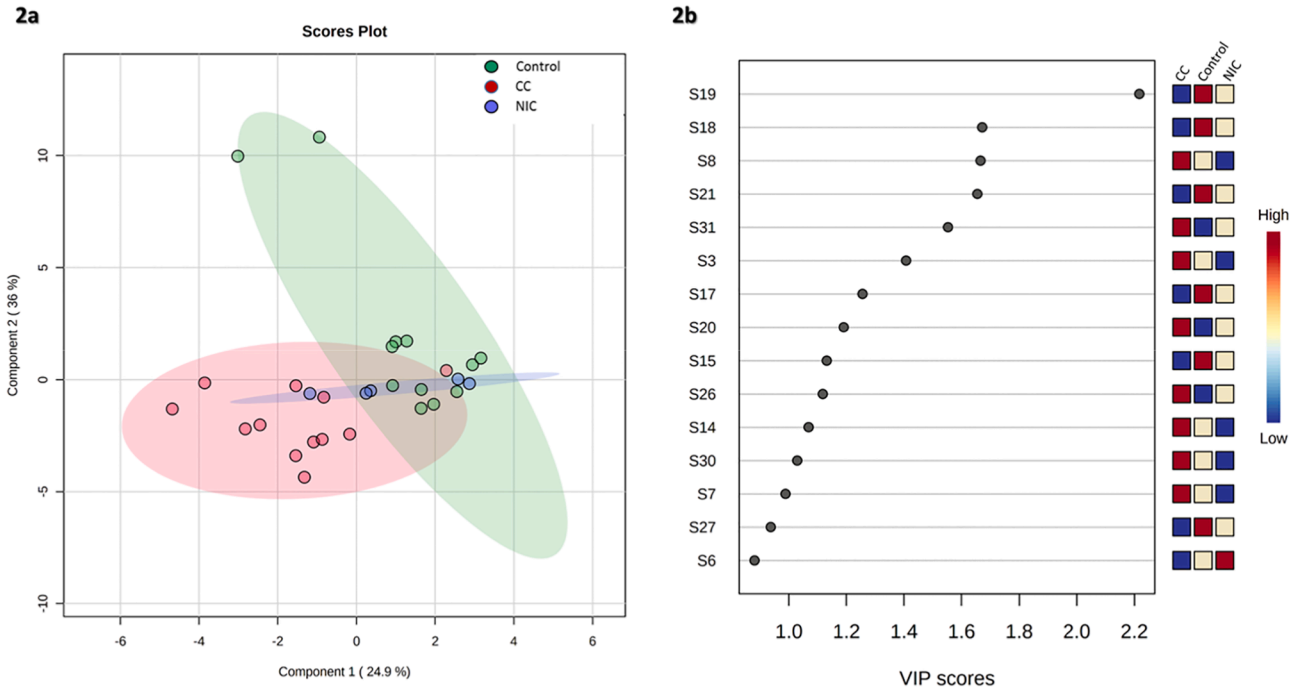

Fig. 2. Partial Least Squares - Discriminant Analysis (PLS-DA). **2a**) Plot of component 1 vs component 2; **2b**) plot of variable importance in projection.

infections are similar and differ from high-risk HPV metabolites and identified that 3 metabolites (5-Oxoprolinate, erythronic acid and N-acetylaspartic acid) are characteristic and differentiate the high-risk group from the control and low-risk group [26]. Chorna et al., demonstrated that changes in urine metabolic patterns may be associated with changes in the vaginal microbiota due to HPV infection [27], thus different types of metabolites, including VOCs can be released in the urine and explain the differences between control women urine and HPV positive women urine.

The principal result of this study is the differentiation between the overall VOCs profiles between the control and CC groups, this technique allows to detect the differences between the metabolites and their concentrations produced due to the pathophysiological process. According to the review of the scientific literature, this is the first study to report the use of an electronic nose for the evaluation of the global urinary VOCs profile of women with CC.

Some metabolomics studies have reported differences in VOCs of controls and CC. A study by Yamamoto et al., demonstrates, by means of

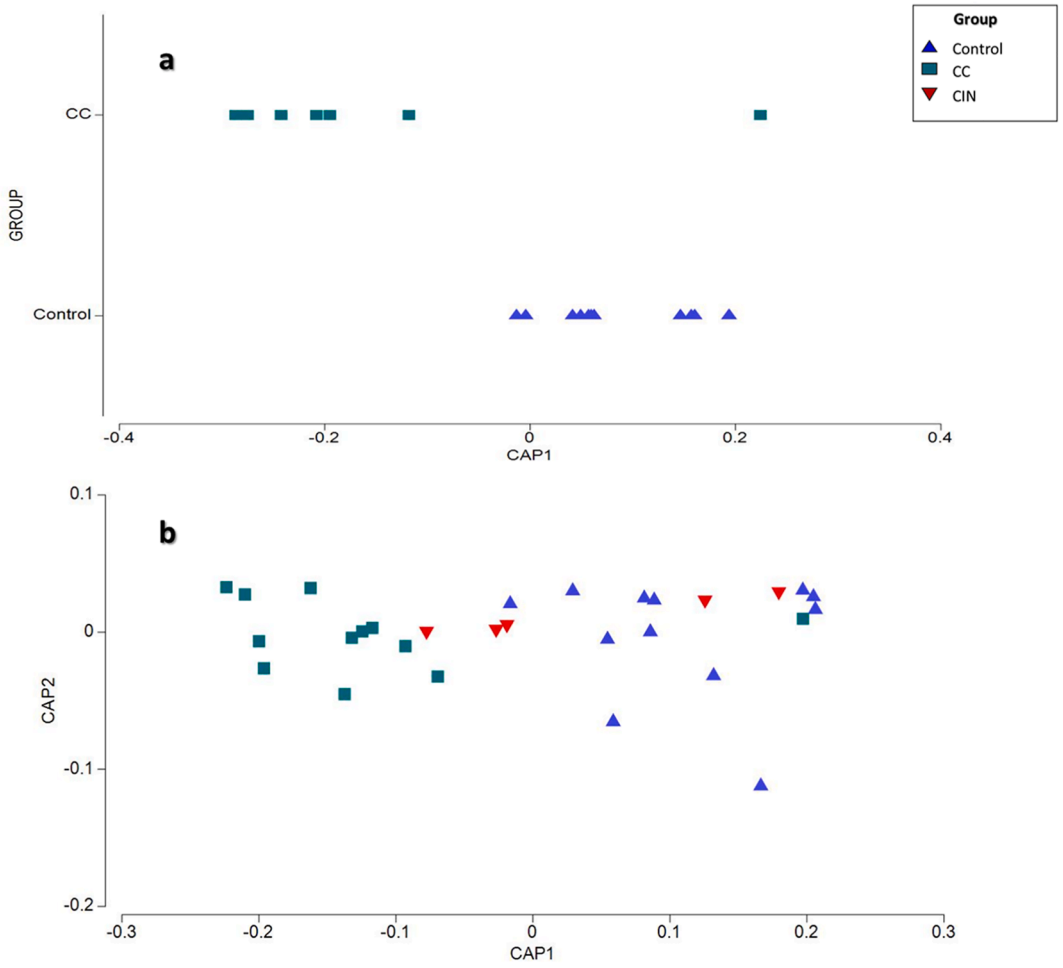

Fig. 3. Canonical Analysis of Principal Coordinates (CAP) of VOCs in control, CC and CIN group. **3a)** plot of control group vs. cervical cancer; **3b)** plot of control group vs. CIN vs. cervical cancer.

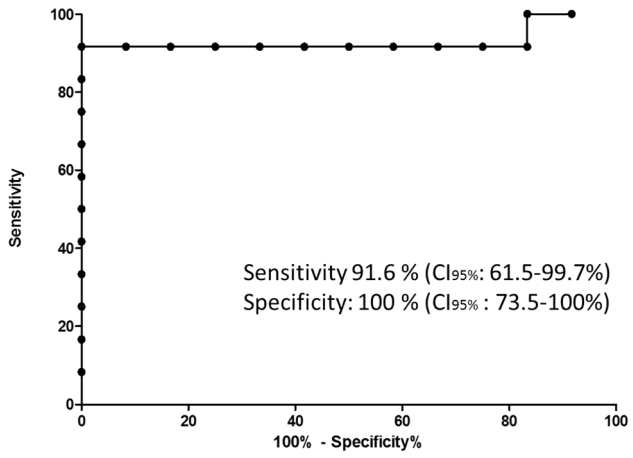

Fig. 4. ROC curve for the screening of CC when using the CAP_1 axis. An AUC of 0.9306 was obtained when using a cut-off point of −0.042.

sniffing dog training, that cervical cancer generates different traces of VOCs in urine, in this study urine samples were collected from 34 patients with cervical cancer, 49 patients with CIN 3, 49 with benign uterine diseases, and 63 healthy volunteers [28]. In all tests, the dog was presented with one positive sample among five samples. The trained dog accurately distinguished the urine sample of a CC patient from those of controls. This study demonstrated that cancer detection by dog sniffing can be a non-invasive and cost-effective screening technique for CC [29].

Other studies carried out in urine have described specific VOCs for the detection of HPV and CC, as is the study carried out by Elia et al., where by means of GC–MS and neural networks they report a signature of VOCs present in the urine of 17 patients with CIN I and 9 healthy women. They found VOCs that differed between these two groups, among which are 3-hexanone, hexanal, 3-Ethylcyclopentanone, Ethanol, 2-(2-ethoxyethoxy), 1-Propene, 1-(ethylthio)-2-methyl, Benzeneacetaldehyde, urea, Dodecane, 4-methyl-, 3,4-Dimethylbenzaldehyde, Naphthalene, 2-methyl, Naphthalene, 1-methyl, 2-Methoxy-4-vinylpheno, Piperitenone, Toluene-2,4-diisocyanate, 5-Methyl-1,3-dihydro-2Hbenzimidazol-2-one, 2-Pentadecanone, 6,10,14-trimethyl, Lidocaine, and Bisphenol A [30]. Some of these metabolites are related from oxidative stress and lipid peroxidation processes and can differentiate between the study groups. This is consistent with the presence of Naphthalenes, Dodecanes, Hexadecanes, Hexanones, Hexanol in our study.

Among the metabolites found in urine by HS-SPME-GC–MS, some have been related to other non-infectious chronic diseases and carcinogenic processes. Such is the case of Octadecane, which has been found in exhaled breath of people suffering from stomach cancer [31] and gastric cancer line cells [32]; Tridecane, has been reported in breath and urine of people with breast cancer [33], urine of people with ovarian cancer [34], the breath of people with lung cancer [35] and in feces of people with NSCLC lung cancer [36]. Nonadecane, which has been reported in the breath of people with breast cancer [37], urine of people

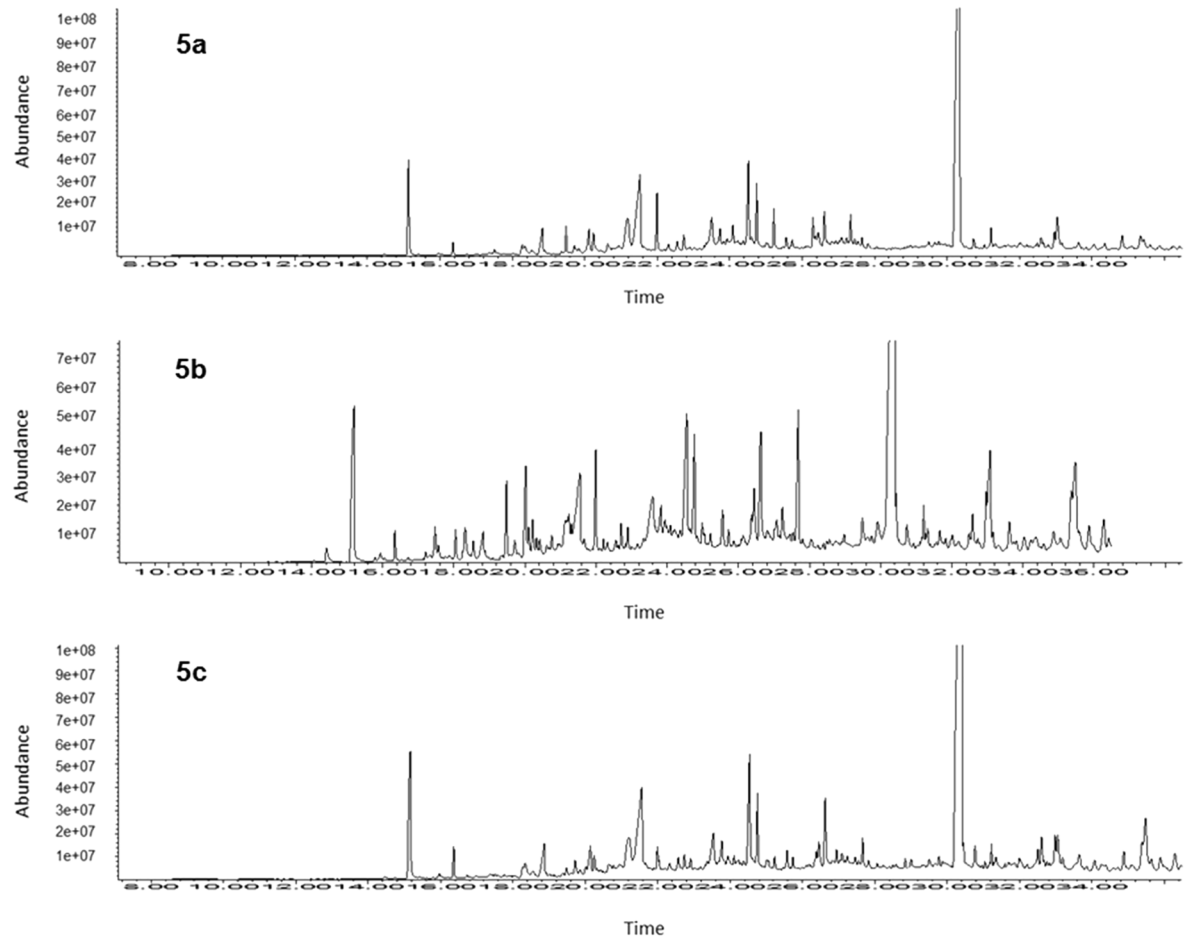

Fig. 5. Chromatograms in urine analyzed by HS-SPME-GC–MS. **5a**) control group urine; **5b**) CIN group urine; **5c**) CC group urine.

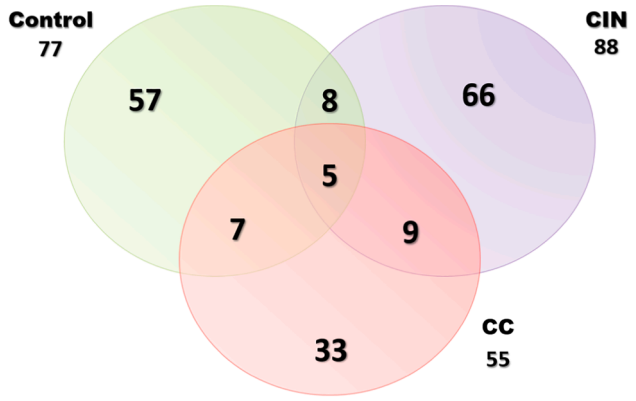

Fig. 6. Venn diagram showing the number of unique and shared metabolites between the three groups.

with ovarian cancer [34], gastric cancer [38]; Trimethyl tetracontane has been reported in the breath of people with lung cancer [39]; Neoclovene in cardiovascular vascular diseases [40]; Phenol, 4,4′-(1,1-dimethyl-3-methylene-1,3-propanediyl)bis- in the urine of people with cellular hepatocarcinoma [41] and Hexadecane in the urine of people with breast cancer, ovarian cancer [34] and breath of people with celiac disorders and ovarian cancer [42].

Rodriguez-Esquivel et al., report VOCs in the female genital area of healthy women and women with CC througt GC–MS, the reported data show several methylated alkane-type VOCs as a result of the lipid membrane and protein oxidation originating from reactions with reactive oxygen species during carcinogenesis [43], these results are similar to those found in our study.

The limitations of our study are associated with the nature of the exploratory study, among the most important were the number of evaluated samples, which was reflected in the results of the CIN group compared to CC and control, so, increasing the population of study remains established as a research perspective and for further validation; also in the analysis of non-targeted metabolomics in GC–MS different VOCs are proposed that may be associated with CC or HPV infection, however, further research is required to support the biological activity from which these metabolites are derived. Another limitation is that due to the number of samples it is not possible to compare and obtain the sensitivity and specificity of the test against the gold standard, however, we consider that the exploratory study fulfilled the objective of demonstrating the clear separation between the control and CC groups, thus establishing the basis for increasing the number of samples and obtaining these parameters. On the other hand, within the strengths, our groups were well characterized in cytological analysis and molecular identification of HPV 16 and 18 genotypes, furthermore, we consider that our proposal of the use of the electronic nose demonstrates the potential of VOCs as biomarkers in urine for the detection of CC. A great advantage of this equipment is its high sensitivity since it detects at trace levels the global VOCs that differentiate CC associated with HPV infection compared to control, the analysis is fast, low-cost and non-invasive. Additionally, urine samples have a high potential to contain biomarkers indicative of physiological state, and to generate a high concentration of volatile compounds that can be used as biomarkers of cancers such as breast cancer [14], renal cell carcinoma [44], preeclampsia [45], renal diseases [46,47], among others and has also been widely studied in the

screening of CC and HPV infections [26]. Analyzing urine would provide biomarkers that change not only because of the transformation of carcinogenic cells but also changes in the local microenvironment. Likewise, several studies report that due to the non-invasive nature of the sample, there is high acceptability by the female population for HPV detection [10,48].

Finally, the WHO's proposed global strategy for the elimination of CC states that "if we want to end the unimaginable suffering caused by cervical cancer, effective and accessible screening and treatment programs in all countries are non-negotiable" [49], cancer screening policies differ markedly between countries, while high-income countries may conduct screening programs for CC, such programs are not recommended in less developed countries, where the healthcare infrastructure is poor [50]. In addition, some factors reduce the effectiveness of programs in these countries, such as cultural barriers, attitudes and beliefs, health environment, education, marital status, income and social support, and immigration and acculturation [51–53]. Cost-effectiveness of screening tests is important for scaling up programmes [49], therefore, the progress of screening methodologies for HPV-associated CC should focus on these aspects, and thus our exploratory study acquires relevance due to the ease of analysis, accessibility and non-invasiveness of the sample collection.

5. Conclusion

This exploratory study demonstrates the feasibility of using olfactory (electronic nose) technology in conjunction with chemometric analysis to assess the differences between healthy women and women with CC associated with HPV infection, thus providing the basis for the development of an accessible, non-invasive, sensitive and specific screening platform for diseases of global concern such as CC and HPV infections. The electronic nose is able to detect volatile organic compounds found in urine at trace levels, thus obtaining a chemical fingerprint of VOCs that is specific to the pathophysiology of cervical cancer. The importance in the generation of accessible, low-cost, fast, sensitive and non-invasive methodologies is of paramount importance in obtaining a timely diagnosis and treatment, for this reason, we consider emphasizing the generation of new olfactory methodologies for cervical cancer screening, which makes the application of the electronic nose ideal for predicting disease. In this manner, subsequent application of confirmatory diagnostic methodologies could improve disease outcomes including in sites of high incidence and low-income settings.

CRediT authorship contribution statement

Lorena Díaz León-Martínez: Conceptualization, Analytical methods, Writing and Editing. **Rogelio Flores-Ramírez:** Conceptualization, Analytical methods, Writing and Editing, Funding. **Carlos Miguel López-Mendoza:** Conceptualization, Analytical methods, Writing and Editing. **Maribel Rodríguez-Aguilar:** Supervision, Validation. **Garima Metha:** Supervision. **Lourdes Zúñiga-Martínez:** Sampling and Editing. **Omar Ornelas-Rebolledo:** Conceptualization, Supervision. **Luz Eugenia Alcántara-Quintana:** Conceptualization, Sampling, Analytical methods, Writing and Editing, Funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] World Health Organization Cancer, Key Statics (2019).
[2] World Health Organization, Human papillomavirus (HPV) (2018).
[3] D. Saslow, D. Solomon, H.W. Lawson, M. Killackey, S.L. Kulasingam, J. Cain, F. A. Garcia, A.T. Moriarty, A.G. Waxman, D.C. Wilbur, American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer, CA Cancer J. Clin. 62 (3) (2012) 147–172.
[4] N. Santesso, R.A. Mustafa, H.J. Schünemann, M. Arbyn, P.D. Blumenthal, J. Cain, M. Chirenje, L. Denny, H. De Vuyst, L.O. Eckert, S.E. Forhan, E.L. Franco, J.C. Gage, F. Garcia, R. Herrero, J. Jeronimo, E.R. Lu, S. Luciani, S.C. Quek, R. Sankaranarayanan, V. Tsu, N. Broutet, World Health Organization Guidelines for treatment of cervical intraepithelial neoplasia 2–3 and screen-and-treat strategies to prevent cervical cancer, International Journal of Gynecology & Obstetrics 132 (3) (2016) 252–258.
[5] T. Malagón, K.D. Volesky, S. Bouten, C. Laprise, M. El-Zein, E.L. Franco, Cumulative risk of cervical intraepithelial neoplasia for women with normal cytology but positive for human papillomavirus: Systematic review and meta-analysis, Int. J. Cancer 147 (10) (2020) 2695–2707.
[6] R.A. Mustafa, N. Santesso, R. Khatib, A.A. Mustafa, W. Wiercioch, R. Kehar, S. Gandhi, Y. Chen, A. Cheung, J. Hopkins, B. Ma, N. Lloyd, D. Wu, N. Broutet, H. J. Schünemann, Systematic reviews and meta-analyses of the accuracy of HPV tests, visual inspection with acetic acid, cytology, and colposcopy, International Journal of Gynecology & Obstetrics 132 (3) (2016) 259–265.
[7] M. Arbyn, E. Paraskevaidis, P. Martin-Hirsch, W. Prendiville, J. Dillner, Clinical utility of HPV–DNA detection: Triage of minor cervical lesions, follow-up of women treated for high-grade CIN: An update of pooled evidence, Gynecologic Oncology 99(3, Supplement) (2005) S7-S11.
[8] S.L. Bedell, L.S. Goldstein, A.R. Goldstein, A.T. Goldstein, Cervical Cancer Screening: Past, Present, and Future, Sexual Medicine Reviews 8(1) (2020) 28-37.
[9] Eliane Rohner, F. Hunter McGuire, Yutong Liu, Quefeng Li, Kate Miele, Samveg A. Desai, HJohn W. Schmitt, Andrea Knittel, Julie A.E. Nelson, Claire Edelman, Vijay Sivaraman, Anna Baker, LaHoma S. Romocki, a. Lisa Rahangdale, J.S. Smith, Racial and Ethnic Differences in Acceptability of Urine and Cervico-Vaginal Sample Self-Collection for HPV-Based Cervical Cancer Screening, Journal of Women's Health 29(7) (2020) 971-979.
[10] H.Y. Shin, B. Lee, S.H. Hwang, D.O. Lee, N.Y. Sung, J.Y. Park, J.K. Jun, Evaluation of satisfaction with three different cervical cancer screening modalities: clinician-collected Pap test vs. HPV test by self-sampling vs. HPV test by urine sampling, J Gynecol Oncol 30(5) (2019) e76-e76.
[11] M. Rodríguez-Aguilar, L. Díaz de León-Martínez, P. Gorocica-Rosete, R. Pérez-Padilla, C.A. Domínguez-Reyes, J.A. Tenorio-Torres, O. Ornelas-Rebolledo, G. Mehta, B.N. Zamora-Mendoza, R. Flores-Ramírez, Application of chemoresistive gas sensors and chemometric analysis to differentiate the fingerprints of global volatile organic compounds from diseases Preliminary results of COPD, lung cancer and breast cancer, Clinica Chimica Acta 518 (2021) 83–92.
[12] K.B. Mendez-Rodriguez, N. Figueroa-Vega, C.A. Ilizaliturri Hernandez, M. Cardona-Alvarado, J.A. Borjas Garcia, C. Kornhauser, J.M. Malacara, R. Flores-Ramirez, F.J. Perez-Vazquez, Identification of metabolic markers in patients with type 2 Diabetes Mellitus by Ultrafast gas chromatography coupled to electronic nose. A pilot study, Biomed Chromatogr (2020), e4956.
[13] L. Yang, Y. Wang, H. Cai, S. Wang, Y. Shen, C. Ke, Application of metabolomics in the diagnosis of breast cancer: a systematic review, J Cancer 11 (9) (2020) 2540–2551.
[14] M. Woollam, M. Teli, P. Angarita-Rivera, S. Liu, A.P. Siegel, H. Yokota, M. Agarwal, Detection of Volatile Organic Compounds (VOCs) in Urine via Gas Chromatography-Mass Spectrometry QTOF to Differentiate Between Localized and Metastatic Models of Breast Cancer, Sci. Rep. 9 (1) (2019) 2526.
[15] G. Johnson, D. Patel, A. Alluhayb, N. Li, C. Shen, T. Webster, Preliminary studies of honey queen bee conditions using Cyranose 320 nose technology, Proceedings of the ACMSE 2018 Conference, Association for Computing Machinery, Richmond, Kentucky, 2018, p. Article 30.
[16] S. Tabbara, A. Saleh, W.A. Andersen, S.R. Barber, P.T. Taylor, C.P. Crum, The Bethesda classification for squamous intraepithelial lesions: histologic, cytologic, and viral correlates, Obstetrics and gynecology 79 (3) (1992) 338–346.
[17] WHO, Human papillomaviruses. IARC working group on the evaluation of carcinogenic risks to humans. IARC Monograph IARC Monograph (90) (2007) 1-636.

[18] Shama Naz, Maria Vallejo, Antonia García, Coral Barbas, Method validation strategies involved in non-targeted metabolomics, J. Chromatogr. A 1353 (2014) 99–105.
[19] Maribel Rodríguez-Aguilar, Lorena Díaz de León-Martínez, Blanca Nohemí Zamora-Mendoza, Andreu Comas-García, Sandra Elizabeth Guerra Palomares, Christian Alberto García-Sepúlveda, Luz Eugenia Alcántara-Quintana, Fernando Díaz-Barriga, Rogelio Flores-Ramírez, Comparative analysis of chemical breath-prints through olfactory technology for the discrimination between SARS-CoV-2 infected patients and controls, Clinica Chimica Acta 519 (2021) 126–132.
[20] K. Hajian-Tilaki, Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation, Caspian J Intern Med 4 (2) (2013) 627–635.
[21] V.-M. Dospinescu, A. Tiele, J.A. Covington, Sniffing Out Urinary Tract Infection—Diagnosis Based on Volatile Organic Compounds and Smell Profile, Biosensors 10 (8) (2020) 83.
[22] H. Kelly, Y. Benavente, M.A. Pavon, S. De Sanjose, P. Mayaud, A.T. Lorincz, Performance of DNA methylation assays for detection of high-grade cervical intraepithelial neoplasia (CIN2+): a systematic review and meta-analysis, Br. J. Cancer 121 (11) (2019) 954–965.
[23] Ramu Rajasekaran, Prakasa Rao Aruna, Dornadula Koteeswaran, Ganesan Bharanidharan, Munusamy Baludavid, Singaravelu Ganesan, Steady-state and time-resolved fluorescence spectroscopic characterization of urine of healthy subjects and cervical cancer patients, J. Biomed. Opt. 19 (3) (2014) 037003, https://doi.org/10.1117/1.JBO.19.3.037003.
[24] Xiao Tan, Xuesen Cao, Jianzhou Zou, Bo Shen, Xiaoyan Zhang, Zhonghua Liu, Wenlv Lv, Jie Teng, Xiaoqiang Ding, Indoxyl sulfate, a valuable biomarker in chronic kidney disease and dialysis, Hemodialysis international, International Symposium on Home Hemodialysis 21 (2) (2017) 161–167.
[25] Qun Liang, Qian Yu, Haikun Wu, Yong-zhi Zhu, Ai-hua Zhang, Metabolite fingerprint analysis of cervical cancer using LC-QTOF/MS and multivariate data analysis, Anal. Methods 6 (12) (2014) 3937–3942.
[26] F. Godoy-Vitorino, G. Ortiz-Morales, J. Romaguera, M.M. Sanchez, M. Martinez-Ferrer, N. Chorna, Discriminating high-risk cervical Human Papilloma Virus infections with urinary biomarkers via non-targeted GC-MS-based metabolomics, PLoS One 13(12) (2018) e0209936.
[27] Nataliya Chorna, Josefina Romaguera, Filipa Godoy-Vitorino, Cervicovaginal Microbiome and Urine Metabolome Paired Analysis Reveals Niche Partitioning of the Microbiota in Patients with Human Papilloma Virus Infections, Metabolites 10 (1) (2020) 36, https://doi.org/10.3390/metabo10010036.
[28] A. Yamamoto, S. Kamoi, K. Kurose, M. Ito, T. Takeshita, S. Kure, K. Sakamoto, Y. Sato, M. Miyashita, The Trained Sniffer Dog Could Accurately Detect the Urine Samples from the Patients with Cervical Cancer, and Even Cervical Intraepithelial Neoplasia Grade 3: A Pilot Study, Cancers 12 (11) (2020) 3291.
[29] H. Guerrero-Flores, T. Apresa-García, Ó. Garay-Villar, A. Sánchez-Pérez, D. Flores-Villegas, A. Bandera-Calderón, R. García-Palacios, T. Rojas-Sánchez, P. Romero-Morelos, V. Sánchez-Albor, O. Mata, V. Arana-Conejo, J. Badillo-Romero, K. Taniguchi, D. Marrero-Rodríguez, M. Mendoza-Rodríguez, M. Rodríguez-Esquivel, V. Huerta-Padilla, A. Martínez-Castillo, I. Hernández-Gallardo, R. López-Romero, C. Bandala, J. Rosales-Guevara, M. Salcedo, A non-invasive tool for detecting cervical cancer odor by trained scent dogs, BMC Cancer 17 (1) (2017) 79.
[30] P. Elia, S. Raizelman, E. Katorza, Y. Matana, O. Zeiri, Z. Boger, M. Cervelli, P. Mariotini, C. Vallone, F. Signore, Biomarkers for the detection of pre-cancerous stage of cervical dysplasia, J. Molecular Biomarkers Diagnosis 6 (6) (2015) 1.
[31] A.L. Jaimesa, C.M. Durána, O.E. Gualdróna, S.R. Ionescub, Stomach Cancer Detection through Exhaled Breath using Biomarkers Analysis, Chem. Eng. 68 (2018).
[32] A. Leiherer, D. Ślefarska, M. Leja, C. Heinzle, A. Mündlein, I. Kikuste, L. Mezmale, H. Drexel, C.A. Mayhew, P. Mochalski, The volatilomic footprints of human HGC-27 and CLS-145 gastric cancer cell lines, Frontiers in Molecular Biosciences 7 (2020) 471.
[33] Michael Phillips, Renee N. Cataneo, Jose Alfonso Cruz-Ramos, Jan Huston, Omar Ornelas, Nadine Pappas, Sonali Pathak, Prediction of breast cancer risk with volatile biomarkers in breath, Breast Cancer Res. Treat. 170 (2) (2018) 343–350.
[34] M. Nakrani, P. Doshi, C. Joshi, M. Joshi, A novel non-invasive method for detection of breast and ovarian cancer using volatile organic compounds from urine sample, (2021).
[35] Yannick Saalberg, Marcus Wolff, VOC breath biomarkers in lung cancer, Clinica Chimica Acta 459 (2016) 5–9.
[36] Andrea Botticelli, Pamela Vernocchi, Federico Marini, Andrea Quagliariello, Bruna Cerbelli, Sofia Reddel, Federica Del Chierico, Francesca Di Pietro, Raffaele Giusti, Alberta Tomassini, Ottavia Giampaoli, Alfredo Miccheli, Ilaria Grazia Zizzari, Marianna Nuti, Lorenza Putignani, Paolo Marchetti, Gut metabolomics profiling of non-small cell lung cancer (NSCLC) patients under immunotherapy treatment, J. Translational Medicine 18 (1) (2020), https://doi.org/10.1186/s12967-020-02231-0.
[37] Michael Phillips, Renee N. Cataneo, Beth Ann Ditkoff, Peter Fisher, Joel Greenberg, Ratnasiri Gunawardena, C. Stephan Kwon, Farid Rahbari-Oskoui, Cynthia Wong, Volatile markers of breast cancer in the breath, Breast J. 9 (3) (2003) 184–191.
[38] J.-D. Hu, H.-Q. Tang, Q. Zhang, J. Fan, J. Hong, J.-Z. Gu, J.-L. Chen, Prediction of gastric cancer metastasis through urinary metabolomic investigation using GC/MS, World Journal of Gastroenterology: WJG 17 (6) (2011) 727.
[39] Abolghasem Jouyban, Djavanshir Djozan, Parastou Mohammadandashti, Aliakbar Alizadeh-Nabil, Hooshangh Ghorbanpour, Maryam Khoubnasabjafari, Mohammad Mohammadzadeh, Co-liquefaction with acetone and GC analysis of volatile compounds in exhaled breath as lung cancer biomarkers, BioImpacts: BI 7 (2) (2017) 99–108.
[40] A.D. Watson, Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: a global approach to lipid analysis in biological systems, J. Lipid Res. 47 (10) (2006) 2101–2111.
[41] Ayman S Bannaga, Frank Kvasnik, Krishna Persaud, Ramesh P Arasaradnam, Differentiating cancer types using a urine test for volatile organic compounds, J. Breath Res. 15 (1) (2020) 017102, https://doi.org/10.1088/1752-7163/abc36b.
[42] Haitham Amal, Da-You Shi, Radu Ionescu, Wei Zhang, Qing-Ling Hua, Yue-Yin Pan, Li Tao, Hu Liu, Hossam Haick, Assessment of ovarian cancer conditions from exhaled breath, Int. J. Cancer 136 (6) (2015) E614–E622.
[43] M. Rodriguez-Esquivel, J. Rosales, R. Castro, T. Apresa-Garcia, O. Garay, P. Romero-Morelos, D. Marrero-Rodriguez, K. Taniguchi-Ponciano, R. Lopez-Romero, H. Guerrero-Flores, B. Morales, M. Mendoza-Rodriguez, D. Mosso-Lara, I. Nunez-Nolasco, P. Castro-Alba, S.E. Meza-Toledo, M. Salcedo, Volatolome of the Female Genitourinary Area: Toward the Metabolome of Cervical Cancer, Arch. Med. Res. 49 (1) (2018) 27–35.
[44] D. Wang, C. Wang, X. Pi, L. Guo, Y. Wang, M. Li, Y. Feng, Z. Lin, W. Hou, E. Li, Urinary volatile organic compounds as potential biomarkers for renal cell carcinoma, Biomed Rep 5 (1) (2016) 68–72.
[45] K.B.M. Rodríguez, L.M.R. Gómez, L.C. Yáñez, R.F. Ramírez, O. Ornelas-Rebolledo, J.A. Borjas-García, F. Pérez-Vázquez, M.R. Aguilar, Application of the electronic nose in predicting preeclampsia in high-risk pregnancies. Pilot study, Arch Med Res (2021).
[46] S. Abdelsattar, Z.A. Kasemy, M. Elsayed, T.A. Elrahem, S.K. Zewain, Targeted metabolomics as a tool for the diagnosis of kidney disease in Type II diabetes mellitus, Br. J. Biomed. Sci. (2021) 1–7.
[47] M. Wang, R. Xie, X. Jia, R. Liu, Urinary Volatile Organic Compounds as Potential Biomarkers in Idiopathic Membranous Nephropathy, Med Princ Pract 26(4) (2017) 375-380.
[48] B.Y. Hernandez, A.C. Tareg, M. Reichhardt, A. Agapito, X. Zhu, A. Sy, A. Yuji, J. Killeen, O. Chan, L.E. Buenconsejo-Lum, Randomized controlled trial evaluating the utility of urine HPV DNA for cervical cancer screening in a Pacific Island population, J. Glob Health Rep 2 (2018), e2018016.
[49] World Health Organization, WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention: web annex A: syntheses of evidence, (2021).
[50] F. Holme, F. Maldonado, O.B. Martinez-Granera, J.M. Rodriguez, J. Almendarez, R. Slavkovsky, P. Bansil, K.A. Thomson, J. Jeronimo, S. de Sanjose, HPV-based cervical cancer screening in Nicaragua: from testing to treatment, BMC public health 20(1) (2020) 495-495.
[51] Sarah Abboud, Emily De Penning, Bridgette Brawner, Usha Menon, Karen Glanz, Marilyn Sommers, Cervical Cancer Screening Among Arab Women in the United States: An Integrative Review, Oncol Nurs Forum 44 (1) (2017) E20–E33.
[52] D.Y. Morales-Campos, S.A. Snipes, E.K. Villarreal, L.C. Crocker, A. Guerrero, M. E. Fernandez, Cervical cancer, human papillomavirus (HPV), and HPV vaccination: exploring gendered perspectives, knowledge, attitudes, and cultural taboos among Mexican American adults, Ethnicity & Health 26 (2) (2021) 206–224.
[53] S.L. Anwar, G. Tampubolon, M. Van Hemelrijck, S.H. Hutajulu, J. Watkins, W. Wulaningsih, P.R.N. for the, Determinants of cancer screening awareness and participation among Indonesian women, BMC Cancer 18 (1) (2018) 208.